Antityrosinase and anti-acne potential of plants traditionally used in the Jongilanga community in Mpumalanga

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Highlights

• Biological activity of plants traditionally used in the Mpumalanga region.

• *Acacia nilotica* showed notable antityrosinase activity (IC_{50}: 12.97 ± 1.07 µg/mL).

• Anti-acne potential of *Ozoroa sphaerocarpa*, *Pterocarpus rotundifolius* and *Schotia brachypetala*.

• Low to moderate antiproliferative effects on non-cancerous human keratinocytes.

Abstract

In South Africa, traditional medicine remains a key source of health care, however majority of the plants used as medicines by traditional health care practitioners have not been investigated for their *in vitro* biological activity. The purpose of the present study was to examine the antityrosinase, anti-acne, antioxidant and cytotoxic potential of 25 ethanolic extracts from 16 different plant families which were collected in the Jongilanga community in the Mpumalanga province. The tyrosinase inhibitory activity was evaluated using the mushroom tyrosinase enzyme to determine potential plant extracts that could treat skin hyperpigmentation. Six of the plant extracts showed a fifty percent inhibitory concentration (IC_{50}) lower than 200 µg/mL, of which *Acacia nilotica* (L.) Delile (12.97 ± 1.07 µg/mL) showed the highest activity, followed by *Schotia brachypetala* Sond. (35.07 ± 0.71 µg/mL) and *Combretum collinum* Fresen. (47.92 ± 1.13 µg/mL). The anti-acne potential of the extracts was evaluated by determining their antibacterial activity against *Cutibacterium acnes*. The highest activity was noted for *Harpagophytum procumbens* (Burch.) with a minimum inhibitory concentration (MIC) of 31.25 µg/mL, followed by *S. brachypetala* (125 µg/mL) and *C. collinum*, *Ozoroa sphaerocarpa* R. Fern & A. Fern and *Pterocarpus rotundifolius* DC. which all showed MIC values of 250 µg/mL. The antioxidant studies revealed that the majority of the plant extracts showed strong DPPH

radical scavenging activity. The 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt (XTT) method was used to assess the cytotoxic effect of the plant extracts, of which only 2 extracts showed strong cytotoxicity against the non-cancerous human keratinocyte (HaCat) cell line namely; *H. procumbens* and *Ipomoea oblongata* Hook. The remaining extracts showed low to moderate cytotoxicity. The promising antityrosinase activity of *A. nilotica*, *S. brachypetala* and *C. collinum* as well as the promising antibacterial activity of *O. spaerocarpa*, *P. rotundifolius* and *S. brachypetala*, together with their low to moderate cytotoxicity against HaCat cells, merits further investigation of these species.

Keywords: Antityrosinase; DPPH; *Cutibacterium acnes*; Cytotoxicity; Human keratinocytes; South African medicinal plants

Abbreviations: HaCat: human keratinocyte; DMEM: Dulbecco's modified Eagle's Medium; XTT: 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt; DPPH: 1, 2-diphenyl-1-picrylhydrazyl; MIC: minimum inhibitory concentration; CFU/mL: colony forming units (CFU) per mL; IC_{50}: fifty percent inhibitory concentrations.

1. Introduction

Over 2000 indigenous plants have been reported for their traditionally usage as medicines in South Africa, which is equivalent to an estimated total of 10% of the floral species in South Africa. Of these traditionally used species, 32% were recorded as being sold in Muthi markets, which included numerous provinces such as Mpumalanga, Gauteng, Limpopo, Eastern Cape and KwaZulu-Natal (Williams et al., 2013). Plants as a source of medicine remains a highly sort after

method of treatment in South Africa. Mander et al. (2007) reported that in the region of 27 million South Africans used plants as a source of medicine, during the time of the study, and it is anticipated that this number has significantly grown since then, as it was reported as a growing industry. Mander et al. (2007) further reported the growth in street trade of medicinal plants in Mpumalanga as well as KwaZulu-Natal. Botha et al. (2004) reported that one market alone in Mpumalanga province, traded in 176 different plant species from 69 different plant families. Widely used medicinal plants in the Mpumalanga province include, *Moringa oleifera* Lam., *Hypoxis hemerocallidea* Fisch., C.A.Mey. & Avé-Lall. (African potato), *Siphonochilus aethiopicus* (Schweif.) B.L. Burtt (African ginger) and *Sutherlandia frutescens* (L.) R.Br. (cancer bush) (Department of Agriculture, 2016). An ethnobotanical study in the Mpumalanga province was conducted by Tshikalange et al. (2016a, 2016b), specifically within villages under the Jongilanga tribal council, to document their usage of plants. The species used in this study were collected with the help of a traditional health practitioner, James Mahore, which have been used for numerous medicinal properties, as documented by James Mahore and from other literature sources (Table 1). In the current study, in collaboration with Tshikalange et al. (2016a, 2016b), some of these documented plants were tested for their medicinal potential, specifically determining whether these traditionally used plants showed antibacterial activity against *Cutibacterium acnes*, were able to inhibit tyrosinase to determine their anti-hyperpigmentation potential, determining their antioxidant activity and whether the plants showed any toxicity towards normal human keratinocytes.

Table 1. Traditional usage of South African plants as documented by James Mahore and various literatures sources.

Scientific name/ family	Common name	Traditional usage
Acacia nilotica (L.) Delile / Fabaceae	Scented-pod acacia	● Bark used in the form of a decoction is used to alleviate coughs by the Zulu community (Behr, 2005). ● A root extract is used against tuberculosis, diarrhea, impotence, hemorrhages, dysentery and gonorrhea (Behr, 2005). ● A leaf extract is used for eye infections, sores, sores related to leprosy, menstrual ailments, ulcers, digestion problems as well as hemorrhages (Behr, 2005). ● Leaves are used as an expectorant to treat pharyngitis and bronchitis. Treatment of wounds and diabetes has also been reported (Al-Fatimi et al., 2007). ● The roots are used to treat various types of cancers particularly those affecting the ears, eyes and testicles as well as the treatment of tuberculosis, liver and spleen diseases (Rather et al., 2015). ● The stem bark is used to treat numerous disorders associated with the skin including ulcerative wounds, leucoderma and burns (Rather et al., 2015).
Agathisanthemum bojeri Klotzsch/ Rubiaceae	Chamaligo (Swahili), Kingobulele (Zaramo)	● A decoction prepared using fresh or dried whole plant is used for vaginal candidiasis (Runyoro et al., 2006). ● The leaves and roots are used in pre-natal care and to treat convulsions and skin diseases (Pakia et al., 2003). ● Swelling of the gonads has been alleviated using the roots (Tshikalange et al., 2016a). ● The flowers are used in the treatment of ringworm infections (Makokha et al., 2017)
Asparagus buchananii Baker/ Asparagaceae	Kakwangasa	● The leaves are used to treat amenorrhoea (Steenkamp, 2003).

Carissa edulis (Forssk.) Vahl/ Apocynaceae	Simple-spined num-num	• Used for skin infections, ectoparasitic diseases, abdominal problems, headaches, and sexually transmitted diseases (STD's) (Tolo et al., 2006). • Used for the treatment of schistosomiasis (Bagla et al., 2012). • Ground root material is used for conditions of the chest (Bagla et al., 2012). • A root infusion is taken orally for stomach aches and coughs as well as in the form of eye drops for cataracts (Mutshinyalo and Malatji, 2012). • Fruits are used for dysentery (Mutshinyalo and Malatji, 2012). • Roots boiled in water are used as an analgesic and to treat malaria (Mutshinyalo and Malatji, 2012). • Malaria and typhoid fever have been treated using fruit and leaf decoctions (Jiofack et al., 2010). • Oral administration of the root is used for rheumatism (Giday et al., 2003). • Root decoctions are administered internally to treat venereal disease, heartburn, arthritis and cancer (Jeruto et al., 2008).
Chlorophytum galpinii (Baker) Kativu/ Asparagaceae	Sephalabanya (Tswana)	• Prevention of umbilical cord infections in newborns (Kirby, 2013).
Combretum collinum Fresen./ Combretaceae	Bicoloured bush willow	• Decoctions are used to treat "Madi" which involves blood disease and pains in the side (Watt and Breyer-Brandwijk, 1962). • Treatment of malaria using root and leaf decoctions (Haerdi, 1964). • The roots are used to aid dysentery and snake bites (Kokwaro, 2009). • Stem bark is used to assist complications associated with rectal prolapse (Hedberg et al., 1982). • Gastroenteritis is treated using fresh roots (Adjanohoun, 1989).

		• Diarrhea and sterility is alleviated using decoctions prepared from the roots, whereas root infusions are used to promote labour and treat pediatric hydroceles (Tabuti et al., 2003). • Ground roots are mixed with a traditional brew to treat pyomyositis (Tabuti et al., 2003).
Combretum molle R.Br.ex G.Don/ Combretaceae	Velvet bush willow Mlama (Nyamwezi)	• An infusion prepared from the leaves is taken orally for chest complaints (Masupa, 2011). • A root decoction is used to relieve constipation, headaches, stomach ailments, vaginal candidiasis, fever, dysentery, swelling and to treat hookworm infections (Masupa, 2011; Runyoro et al., 2006). • Fruit are used to aid in childbirth (Watt and Breyer-Brandwijk, 1962). • Leaves are used for wound dressing; antidiarrheal; anthelmintic; dropsy; chest complaints; and as an aid in childbirth (Drummond and Coates-Palgrave, 1973; Haerdi, 1964; Kerarho and Adam, 1974; Kokwaro, 2009). • Treatment of angina and stomach problems using the stem bark (Kerarho and Adam, 1974; Watt and Breyer-Brandwijk, 1962). • A wound dressing is prepared from the roots. The roots have also been used for hookworm infections, snakebites, leprosy, general body swellings, fever, stomach pains, constipation, sterility and abortion (Chhabra et al., 1989; Drummond and Coates-Palgrave, 1973; Kokwaro, 2009; Watt and Breyer-Brandwijk, 1962).
Crabbea velutina S.Moore/ Acanthaceae	Mkunga (Zigua) Kikulagembe (Zaramo)	• A tea prepared from the ground leaves is taken for worms (Tabuti et al., 2003). • A decoction of the whole plant is taken orally for candidiasis (Runyoro et al., 2006). • Stomach aches have been alleviated using roots and leaves (Gemedo-Dalle et al., 2005). • A tea of the leaves is prepared for the treatment of malaria (Meragiaw and Asfaw, 2014). • The root and leaf decoction and infusion are drank and used as a wash for the treatment of scabies (Geissler et al., 2002).

Crotalaria burkeana Benth./ Fabaceae	Rattle bush	• The plant is consumed as a nutritional source of micronutrients (Steyn et al., 2001).
Ficus burkei (Miq.) Miq./ Moraceae	Common wild fig Mutechani/ Murovamhur	• Latex from the leaves is applied topically to treat numerous skin conditions such as skin rashes, eczema, psoriasis, skin cancer and genital warts (Chinsembu et al., 2015).
Harpagophytum procumbens (Burch.) DC.ex Meisn./ Pedaliaceae	Devil's claw	• Secondary root tubers are used to aid with fever, blood diseases, digestive disorders, and arthritic and rheumatic conditions (Clarkson et al., 2003). • Roots are used as an analgesic and as an ointment for sores, ulcers and boils (Smithies, 2006). • The fresh tubers are prepared as an ointment for sores, ulcers, boils and cancerous growths (Mncwangi et al., 2012). • Secondary tubers are powdered and used as a wound dressing directly, or mixed with animal fat or petroleum jelly to treat burns and wounds (Mncwangi et al., 2012).
Helichrysum pallidum DC./ Asteraceae	Mpetso	• The roots are used for penile sores (Tshikalange et al., 2016a). • The whole plant is used to treat tuberculosis (Seleteng et al., 2015).
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall/ Hypoxidaceae	Star flower	• Corms are used to treat bladder disorders and testicular tumors (Steenkamp and Gouws, 2006). • Used as an anti-HIV agent and related urinary tract infections (Naidoo et al., 2013). • Used for the treatment of skin conditions such as; dermatitis, acne, wounds and rashes; diseases including; HIV, cancer, diabetes and high blood pressure and general ailments which include headaches, stomach problems, dysentery, dizziness, burns and mental disorders (Ncube et al., 2013). • Combinations of corms and leaves with other plant species is used to treat internal and external sores and sexually transmitted diseases like gonorrhea and genital warts (de Wet et al., 2012).

Ipomoea crassipes Hook./ Convolvulaceae	Wildepatata	• Roots are used as an anti-HIV agent (Tshikalange et al., 2016a) • Decoctions and infusions of the whole plant are used to treat dysentery (Olajuyigbe and Afolayan, 2012).
Ipomoea oblongata E.Mey.ex Choisy/ Convolvulaceae	Dema	• A decoction of the bulbs are used for asthma and high blood pressure (Tshikalange et al., 2016a). • Cancer, stomach problems and swollen feet have been treated using the roots (Seleteng et al., 2015). • Root infusions are used to soak swollen limbs (Komoreng et al., 2017).
Leonotis nepetifolia (L.) R.Br./ Lamiaceae	Klip dagga	• Used for the treatment of bronchial asthma, diarrhea, fever, malaria, menstrual pain, common colds and to alleviate coughing (Sobolewska et al., 2012). • Flowers are used in India for wound healing, while the seeds are used to aid the treatment of burns and to lighten scars (Sobolewska et al., 2012). • The seeds are used to treat burns (Ashish et al., 2011).
Ozoroa sphaerocarpa R.Fern & A.Fern/ Anacardiaceae	Currant resin tree Xinungu mafi	• A decoction or infusion of the whole plant is used to induce lactation and to heal wounds (Tshikalange et al., 2016a).
Pterocarpus angolensis DC./ Fabaceae	Transvaal teak Mrhotso	• A decoction of the roots are used for heartburn, stomach problems and to induce vomiting (Tshikalange et al., 2016a). • The ground bark is used to treat impotence (Semenya et al., 2013). • The prevention of diarrhea using a bark and root infusion (Semenya and Maroyi, 2012). • A 1:1 decoction with *Athrixia phylocoides* is used to clean wounds (Amusan et al., 2002).
Pterocarpus rotundifolius (Sond.) Druce/ Fabaceae	Round-leaved bloodwood Nxelele	• Roots are used to treat anemia, venereal and kidney diseases (Kamuhabwa et al., 2000). • The stem bark is used for general ailments such as headaches, stomach aches, diarrhea, mouth ulcers and rashes. It is also used to treat malaria and gonorrhea (Samie et al., 2009).

		• The sap is used to treat ringworm infections, ulcers, malaria, urinary schistosomiasis and skin inflammation (Samie et al., 2009). • Ear aches have been alleviated using a bark infusion (Sigidi et al., 2016). • Root infusions are used for infertility, eye infections, wounds, and psoriasis (Sigidi et al., 2016). • Boiled extracts are taken orally for menorrhagia (Sigidi et al., 2016)
Schotia brachypetala Sond./ Fabaceae	African walnut	• The plant is traditionally used to steam the face for pimples, wash the body to reduce body swelling and to treat ulcers (Van Wyk and Gericke, 2000). • Bark is used to treat dysentery, heartburn, diarrhea and nervous conditions (Steenkamp et al., 2005). • The bark extract is used to treat heartburn, pimples and diarrhea (Coopoosamy and Naidoo, 2012).
Searsia transvaalensis (Engl.) Moffett/ Anacardiaceae	River firethorn currant	• The fruit is consumed as a food source in Swaziland (Long, 2005).
Strychnos madagascariensis Poir./ Loganiaceae	Black monkey orange Nkwakwa	• A decoction or infusion of the roots induces vomiting (Tshikalange et al., 2016a). • Burns, sores and ringworm infections are treated using the leaves (Nciki et al., 2016). • The bark is used for sores (de Wet et al., 2010). • Diarrhea is treated using an infusion prepared using the roots, bark and leaves (Maroyi, 2011) • The leaf infusion is used as eye drops for painful eyes (Maroyi, 2011) • The leaves are mixed with the fruit of *Strychnos spinose* to treat ringworm infections (Maroyi, 2011).
Synadenium cupulare L.C. Wheeler/ Euphorbiaceae	Dead-man's tree	• The whole plant is used for the treatment of septic wounds, sores, bruises, backache, rheumatic joints, ulcers, and swelling (Ndhlala et al., 2013).

		• A paste is prepared from the leaves and used as a dressing (Ndhlala et al., 2013). • The fresh latex collected from the leaves and stems is used as an application on wounds (Magwede et al., 2016).
Triaspis hypericoides Burch./ Malpighiaceae	Small shieldfruit Klapperbossie (Afrikaans)	• The Zulu's and Swazi's soak the roots to increase virility (Schmidt et al., 2002).
Vangueria infausta Burch./ Rubiaceae	Wild medlar Xinyathelo	• Root infusions are used to treat malaria (Bapela et al., 2014). • Toothaches, ringworm infections, malaria and pneumonia have been treated using a leaf and root infusion (Mthethwa et al., 2014). • The fruits have been used to treat malaria and to alleviate genital swelling, menstrual and uterine problems as well as a dressing for wounds (Babiaka et al., 2015). • A decoction of the roots is used for snake bites and as a snake repellent (Tshikalange et al., 2016a). • Diarrhea has been treated using an infusion or maceration prepared from the root or bark (de Wet et al., 2010). • Used as an oral wash to treat candidiasis (Chinsembu and Hedimbi, 2010).

2. Materials and methods

2.1 Materials, chemicals and reagents

The human keratinocyte (HaCat) cell line was donated by Dr. Lester Davids from the Department of Human Biology, University of Cape Town. The Dulbecco's modified Eagle's Medium (DMEM), phenol-red trypsin-EDTA (0.25 %), phosphate buffered saline, fetal bovine serum, antibiotics and antifungal agents were purchased from ThermoFisher Scientific (Johannesburg, South Africa). Cell culture plates and flasks were sourced from Lasec SA (Pty) Ltd. (Midrand, South Africa). Pure cultures of *C. acnes* (ATCC 11287) were purchased from Anatech Analytical Technology (Johannesburg, SA). Anaerocult A was purchased from Merck (Pty) Ltd. The Cell Proliferation Kit II, 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxyanilide salt (XTT), and other chemicals and reagents, including; 1, 2-diphenyl-1-picrylhydrazyl (DPPH), dimethyl sulfoxide (DMSO), actinomycin D (purity $\geq$ 95 %), ascorbic acid (purity $\geq$ 99 %), kojic acid (purity $\geq$ 98 %), tetracycline (purity $\geq$ 98 %) and the mushroom tyrosinase enzyme, were purchased from Sigma-Aldrich (Johannesburg, South Africa).

2.2 Methods

2.2.1 Plant collection and extraction

Plant species were collected in the Mpumalanga province in the Jongilanga community. A voucher herbarium specimen was pressed and deposited for reference at the HGWJ Schweickerdt Herbarium (University of Pretoria) Table 2. The South African National Biodiversity Institute (SANBI) assisted in the identification of the plant species. Plant names were confirmed using The Plant List (http://www.theplantlist.org). Processing of the dried plant material included grinding to a powder and macerating the material (20 g) in absolute ethanol (300 mL) for 48 h

Table 2. Medicinal plants extracted, plant parts used and herbarium specimen numbers

Scientific name	PRU number	Plant part
Acacia nilotica (L.) Delile	BMC 117174	Root bark
Agathisanthemum bojeri Klotzsch	BCM 119330	Leaves and stems
Asparagus buchananii Baker	BMC 119329	Roots
Carissa edulis Vahl	BCM 119351	Roots
Chlorophytum galpinii (Baker) Kativu	BCM 120516	Whole plant
Combretum collinum Fresen.	BCM 117156	Bark
Combretum molle R.Br.ex G.Don	BC 194	Roots
Crabbea velutina S.Moore	BCM 120517	Roots
Crotalaria burkeana Benth.	BCM 117184	Stem, Leaves and roots
Ficus burkei (Miq.) Miq.	BC 89	Roots
Harpagophytum procumbens (Burch.) DC. ex Meisn.	BC 199	Roots
Helichrysum pallidum DC.	BCM 119348	Leaves and stems
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall	BC 42	Bulb
Ipomoea crassipes Hook.	BC 162	Stems
Ipomoea oblongata E.Mey. ex Choisy	BCM 119362	Root bark
Leonotis nepetifolia (L.) R.Br.	BC 95	Stems
Ozoroa sphaerocarpa R. Fern & A. Fern	BCM 119359	Leaves
Pterocarpus angolensis DC.	BCM 117169	Root bark
Pterocarpus rotundifolius (Sond.) Druce	BCM 117164	Leaves
Schotia brachypetala Sond.	BCM 119370	Root bark
Searsia transvaalensis (Engl.) Moffett	BC 195	Root bark
Strychnos madagascariensis Poir.	BCM 117163	Bark (big root)
Synadenium cupulare L.C. Wheeler	BC 109	Bark
Triaspis hypericoides Burch.	BCM 120512	Whole plant
Vangueria infausta Burch.	BCM 117162	Roots

with continuous agitation. Solid materials were separated from the filtrate using a Buchner funnel (Whatman No.1 filter paper). Crude extracts were prepared by rotary evaporation (Büchi Rotavapor R-200).

2.2.2 Cell culture

The HaCat cells were maintained in DMEM using T75 cell culture flasks until a confluent monolayer formed. The cell culture media was supplemented with 1% antibiotics containing penicillin (100 U/mL)-streptomycin (100 µg/mL), 1% fungizone (250 µg/L) and 10% fetal bovine serum. A humidified incubator, set at 5% CO_2 and 37°C, was used to grow the cells. Sub-culturing of cells was performed, using trypsin, after a confluent monolayer had formed.

2.2.3 Cytotoxicity assay

The cytotoxic effect of the plant extracts on the HaCat cells was measured using the XTT cell viability reagent. The assay was performed according to a method published by Berrington and Lall (2012). Cells were seeded in microtiter culture plates at a concentration of 1×10^5 cells/mL and incubated overnight at 37ºC and 5% CO_2 to allow for the cell adherence. Stock solutions of the extracts (20 mg/mL in DMSO) and actinomycin D (positive control) (1 mg/mL in distilled water) were prepared. The final concentrations of the extracts and actinomycin D ranged from 3.125-400 and 3.9×10^{-4}-0.05 µg/mL respectively in the 96-well plates. Medium was included as a control and DMSO at 2% (vehicle control). Cells were treated with the different samples and controls for 72 h after which 50 µl of the XTT reagent was added to each of the wells. Duplicate plates, as described above, were also prepared, however these plates contained no cells as they were used as colour controls for the XTT reagent. Two hours after the addition of the XTT reagent, the absorbance of the samples was measured at $OD_{490\ nm}$, with a reference wavelength

set at $OD_{690\ nm}$ using a BIO-TEK power-wave XS plate reader (Analytical and Diagnostic Products CC, Roodepoort, South Africa). Each sample was performed in triplicate. The percentage cell viability, after treatment with each of the samples, was calculated using the following equation:

$$(\%\ viability) = \frac{Absorbance\ sample}{Absorbance\ control\ (DMSO)} \times 100$$

Where $Absorbance_{control}$ is the absorbance of (XTT + vehicle control) – (blank values of the vehicle control) and $Absorbance_{sample}$ is the absorbance of (XTT + sample or the positive control) – (blank values of sample or the positive control). The fifty percent inhibitory concentration (IC_{50}) values were determined using GraphPad Prism 4.

2.2.4 DPPH radical scavenging activity

The DPPH radical scavenging activity of the plant extracts was determined using the methods described by Berrington and Lall (2012). Stock concentrations of the plant extracts were prepared to 2 mg/mL (in ethanol). This was followed by a two-fold serial dilution of the extracts in a 96-well plate, by the addition of 20 µL of extract stock solution to 200 µL of distilled water to obtain a concentration range of 0.78-100 µg/mL. The positive control, ascorbic acid was prepared the same as the plant extracts. Ethanol was used as the negative control. Subsequently, 90 µL of 0.04 M DPPH solution (in ethanol) was added to plant extracts and ascorbic acid wells. To account for interference by the plant extracts, blank plates were prepared by adding 90 µL of ethanol in place of DPPH. Plates were then incubated in the dark for 30 min. Following incubation, the absorbance was measured $OD_{515\ nm}$ using a BIO-TEK PowerWave XS plate reader. The percentage DPPH radical scavenging activity was calculated as follows:

$$\%\ antioxidant\ activity = \frac{(Absorbance\ control - Absorbance\ sample)}{Absorbance\ control} \times 100$$

Where Absorbance $_{control}$ is the (absorbance of the negative control) – (absorbance of the ethanol blank); Absorbance $_{sample}$ is the (absorbance of the extract or positive control) – (absorbance of the extract or positive control blank). The IC_{50} values were calculated using GraphPad Prism 4 from triplicate values.

2.2.5 Tyrosinase inhibitory activity

The tyrosinase inhibitory activity was determined using the methods described by Curto et al., 1999. Plant extracts were prepared to a stock concentration of 20 mg/mL in DMSO. Subsequent dilution of the extracts were in a 24-well plate by the addition of 30 µL in 970 µL of potassium phosphate buffer (pH 6.5). Extracts were then serially diluted two-fold to obtain a concentration range of 1.5-200 µg/mL. Kojic acid, the positive control was prepared in a similar manner. A negative control, 1% v/v DMSO, was included. The inhibitory activity of the extracts was determined by the addition of 70 µL of each plant extract dilution to 30 µL of tyrosinase enzyme (333 units/mL in phosphate buffer) and incubating for 5 min at 25°C. The reaction was then initiated by the addition 110 µL of the substrate, L-tyrosine (2 mM). The absorbance values were determined kinetically over a period of 30 min at $OD_{492\ nm}$ using a BIO-TEK Power-Wave XS plate reader. The percentage tyrosinase inhibition was calculated using the following equation:

$$\%\ inhibition = 100 - (\frac{Absorbance\ sample}{Absorbance\ control}) \times 100$$

Where Absorbance $_{control}$ is the (absorbance of DMSO at time 30) – (absorbance of DMSO at time 0); Absorbance $_{sample}$ is the (absorbance of the extract or positive control at time 30) – (absorbance of the extract or positive control at time 0). The IC_{50} values were calculated using GraphPad Prism 4 from triplicate values.

2.2.6 Antimicrobial susceptibility of Cutibacterium acnes to extracts

The susceptibility of *C. acnes* to antimicrobial agents was determined using a method described by Eloff, 1998. Pure bacterial cultures of *C. acnes* were maintained on sterile nutrient agar for 72 h in anaerobic jars containing anaerocult A. Cultures were then inoculated in sterile nutrient broth using a sterile inoculation loop to a final concentration of 1.5 x 10^8 colony forming units (CFU) per mL (CFU/mL). Extracts were tested at a concentration range of 3.91-500 µg/mL by preparing a 2 mg/mL stock concentration in 10% DMSO. Each sample was tested in triplicate. In a 96-well plate, a two-fold serial dilution of extracts were made by adding 100 µL of the stock concentration to 100 µL of nutrient broth. Serial dilutions of the positive control, tetracycline, were prepared in a similar manner with a stock concentration of 0.2 mg/mL to yield a concentration range of 0.78 - 100 µg/mL. The *C. acnes* inoculum was combined with the extracts by the addition of 100 µL of the inoculated nutrient broth with serially diluted extracts and positive control. Control included the negative control (10% DMSO) and untreated bacterial control. Plates were then incubated at 37°C in anaerobic conditions for 72 h. Bacterial growth was detected with the addition of 20 µL of the PrestoBlue reagent to determine minimum inhibitory concentration (MIC) for each extract. Visual interpretation of the colour reaction, from blue to pink, was observed after 1 h.

2.2.7 Statistical analysis

Data obtained for each experiment was a result of three independent replicates (n=3). Results were reported as the mean values ± standard deviation (SD). The IC_{50} values for the cytotoxicity, antioxidant and antityrosinase activity were calculated using nonlinear regression analysis of the sigmoidal dose response curves using GraphPad Prism 4. For the antibacterial activity, the minimum inhibitory concentration (MIC) was visually interpreted.

3. Results and discussion

3.1 Cytotoxicity assay

The present study is the first to report the effects of the plant extracts evaluated on a non-cancerous skin cell line, with the exception on *C. molle* (Table 3). As described by Kuete and Efferth (2015) plant extracts tested against non-cancerous cell lines, have strong, moderate, low or no cytotoxic effects when the IC_{50} is < 100 µg/mL, 100 µg/mL < IC_{50} < 300 µg/mL, 300 µg/mL < IC_{50} < 1000 µg/mL and IC_{50} > 1000 µg/mL, respectively. The ethanolic root extract of *C. molle* showed no cytotoxicity up to a concentration of 400 µg/mL on the HaCat cell line. A study by Rademan et al. (2019) reported an IC_{50} of 45.9 ± 7.0 and 104.3 ± 0.3 µg/mL on HaCat cells by the ethanolic fruit and leaf extracts respectively. Furthermore, this study indicated that the ethanolic fruit and leaf extracts showed promising activity on the A431 and UCT-Mel 1 skin cancer cell lines. As the ethanolic root extract of *C. molle* showed no cytotoxicity against the non-cancerous HaCat cells, the antiproliferative activity of the root extract against skin cancer cell lines should be considered. This could potentially result in a high safety margin should the extract show significant cytotoxicity, which would be selectively more active towards skin cancer without normal keratinocyte toxicity.

Table 3. Investigated biological activity of traditionally used species found within the Jogilanga region

Plant extracts	Antityrosinase	DPPH[a]	HaCat[b]	Antibacterial
		IC_{50}[c] ± SD[d] in µg/ml		MIC[e] in µg/ml
Acacia nilotica (L.) Delile	12.97 ± 1.07	3.60 ± 0.33	188.00 ± 8.70	500
Agathisanthemum bojeri Klotzsch	NI	46.60 ± 3.90	NI	NI
Asparagus buchananii Baker	NI	87.68 ± 14.20	NI	NI
Carissa edulis (Forssk.) Vahl	NI	57.10 ± 6.60	NI	NI
Chlorophytum galpinii (Baker) Kativu	NI	54.50 ± 6.20	NI	NI
Combretum collinum Fresen.	47.92 ± 1.13	1.14 ± 1.14	153.00 ± 3.90	250
Combretum molle R.Br.ex G.Don	NI	2.80 ± 0.70	NI	NI
Crabbea velutina S.Moore	NI	8.80 ± 3.50	NI	NI
Crotalaria burkeana Benth.	NI	98.30 ± 9.00	NI	NI
Ficus burkei (Miq.) Miq.	NI	6.70 ± 0.90	106.10 ± 6.30	NI
Harpagophytum procumbens (Burch.) DC. ex Meisn.	NI	9.10 ± 0.02	44.60 ± 4.50	31.25
Helichrysum pallidum DC.	NI	8.50 ± 2.50	NI	NI
Hypoxis hemerocallidea Fisch., C.A.Mey. & Avé-Lall	NI	23.92 ± 1.44	NI	NI
Ipomoea crassipes Hook.	NI	20.60 ± 2.60	219.60 ± 0.77	NI
Ipomoea oblongata E.Mey. ex Choisy	190 ± 1.20	40.60 ± 2.90	52.70 ± 8.60	NI
Leonotis nepetifolia (L.) R.Br.	NI	12.90 ± 0.40	NI	NI
Ozoroa sphaerocarpa R. Fern & A. Fern	NI	9.10 ± 0.10	NI	250
Pterocarpus angolensis DC.	NI	18.83 ± 2.11	195.90 ± 4.80	NI

Pterocarpus rotundifolius (Sond.)	124.40 ± 2.03	8.68 ± 1.69	NI	250
Schotia brachypetala Sond.	35.07 ± 0.71	2.01 ± 0.13	130.50 ± 4.80	125
Searsia transvaalensis (Engl.) Moffett	NI	3.50 ± 0.20	NI	NI
Strychnos madagascariensis Poir.	NI	9.60 ± 2.14	NI	NI
Synadenium cupulare L.C. Wheeler	NI	105.50 ± 8.70	306.01 ± 15.20	NI
Triaspis hypericoides Burch.	NI	52.10 ± 20.70	NI	NI
Vangueria infausta Burch.	52.81 ± 1.17	32.00 ± 0.45	NI	NI
Positive control[f]	1.38 ± 0.05	1.90 ± 0.05	0.01 ± 2.30	0.78

NI: No inhibition at the highest tested concentration; [a] 2, 2-diphenyl-1-picrylhydrazyl radical; [b] Human keratinocyte cells; [c] Fifty percent inhibitory concentration; [d] Standard deviation; [e] Minimum inhibitory concentration; [f] Positive control for tyrosinase inhibition (kojic acid), DPPH radical scavenging activity (ascorbic acid); cytotoxicity (actinomycin D), and for *Cutibacterium acnes* (Tetracycline). Values are represented as Mean±SD.

The majority of the other plant extracts (*A. bojeri, A. buchananii, C. edulis, C. galpinii, C. velutina, C. burkeana, C. schinzii, H. pallidum, H. hemerocallidea, L. nepetifolia, O. sphaerocarpa, P. rotundifolius, S. transvaalensis, S. madagascariensis, T. hypericoides,* and *V. infausta*) did not show cytotoxicity on the HaCat cells at a concentration of 400 µg/mL. Considering the traditional use of some of these plants (*C. edulis, C. molle, H. hemerocallidea, L. nepetifolia, O. sphaerocarpa, S. madagascariensis,* and *V. infausta*) for boils, burns, pimples, rashes, skin infections, sores, and wounds, the results of the present study validated the use of these species in a topical dosage form (Table 1).

Nine of the plant extracts namely; *A. nilotica* (188.0 ± 8.7 µg/mL), *C. collinum* (153.0 ± 3.9 µg/mL), *F. burkei* (106.1 ± 6.3 µg/mL), *H. procumbens* (44.6 ± 4.5 µg/mL), *I. crassipes* (219.6 ± 0.77 µg/mL), *I. oblongata* (52.7 ± 8.6 µg/mL) *P. angolensis* (195.9 ± 4.8 µg/mL), and *S. cupulare* (306.01 ± 15.2 µg/mL) and *S. brachypetala* (130.5 ± 4.8 µg/mL), showed moderate cytotoxicity, however, these plants have been traditionally used as a topical application to the

skin. In previous reports by Kamuhabwa et al. (2000) and Sigidi et al. (2016) the methanolic extract prepared from the roots and an aqueous bark extract of *P. angolensis* showed antiproliferative activity against the A431 (100 µg/mL) and MeWo (100 µg/mL) skin cancer cell lines. When considering that the IC_{50} value found in this study on the non-cancerous skin cell line was almost double that of the active concentrations reported against the skin cancer cell lines, *P. angolensis* should be strongly considered for further evaluation against skin cancer. *C. collinum* has been previously evaluated on a skin cancer cell line, SK-MEL-28. A study by Abreu et al. (1999) described that the methanolic root extract of *C. collinum* had no antiproliferative activity on the SK-MEL-28 melanoma skin cancer cell line up to a concentration of 20 µg/mL. The ethanolic extracts of the roots of *H. procumbens* and the root bark of *I. oblongata* showed the highest cytotoxicity on HaCat cells, with IC_{50} values lower than 100 µg/mL. Due to this cytotoxic effect, these extracts should be evaluated with caution for other bioactivities where topical application will be considered in the future.

3.2 Antioxidant activity

The production of reactive oxygen species (ROS) in the skin is a result of continuous exposure to external environmental factors, which contributes towards an increase in oxidative stress. Oxidative stress has been linked to the onset of many inflammatory diseases such as acne vulgaris, cancer and skin disorders such as vitiligo and hyperpigmentation. Therefore, antioxidants play a vital role in the treatment regimens of these diseases. Previous studies have confirmed the accumulation of synthetic antioxidants in the body, which has led to an increased use of antioxidants from a natural origin, as these are considered to be safer alternatives to synthetic antioxidants. The antioxidant activity of botanical extracts is speculated to be due to the constant exposure of plants to endogenous and exogenous ROS. Therefore, they have evolved to

produce antioxidants for protection against oxidative damage (Athwal, 2015; Brewer, 2011; Hasan et al., 2009; Lambrechts et al., 2018).

According to Phongpaichit et al. (2007) an extract is considered to have a high antioxidant capacity if it possesses an IC_{50} between 10-50 µg/mL and lower, a moderate antioxidant capacity with an IC_{50} value between 50-100 µg/mL and a weak antioxidant capacity with an IC_{50} above 100 µg/mL (Jadid et al., 2017; Phongpaichit et al., 2007).

The DPPH scavenging effects of the extracts are presented in Table 3. Noteworthy antioxidant activity was observed for *A. bojeri*, *H. hemerocallidea*, *I. oblongata* and *V. infausta* with IC_{50} values below 50 µg/mL. Extracts prepared from *A. nilotica*, *C. collinum*, *C. molle*, *C. velutina*, *F. burkei*, *H. procumbens*, *H. pallidum*, *I. crassipes*, *L. nepetifolia*, *O. sphaerocarpa*, *P. angolensis*, *P. rotundifolius*, *S. brachypetala*, *S. transvaalensis* and *S. madagascariensis* exhibited activity that was comparable to that of the positive control, L-ascorbic acid. Moderate antioxidant activity was observed for *A. buchananii*, *C. edulis*, *C. galpinii*, *C. burkeana*, and *T. hypericoides* with IC_{50} values between 50 and 100 µg/mL.

Antioxidative phenolics found in plants have been linked to high antioxidant potential. These phenolics are divided into four major groups, the first being phenolic acids such as gallic acid, protochatechuic acid, caffeic acid and rosmarinic acid. Phenolic acids are known for their antioxidant capacity as a result of their ability to trap free radicals. The second and third group of antioxidant plant phenolics are phenolic diterpenes, such as carnosol and carnosic acid, as well as flavonoids such as catechin and quercetin. Flavonoids are able to chelate metals and scavenge free radicals, contributing to its antioxidant capacity. Volatile oils such as eugenol, carvacrol, thymol and menthol are the fourth antioxidant group. Although these compounds are known for their effective antioxidant activity, a balance should be maintained between the proportion of

antioxidants and oxidants as oxidants play a vital role in the production of new skin cells (Brewer, 2011; Thibane et al., 2018). This is essential for disorders such as acne vulgaris, skin ageing and wound repair.

3.3 Tyrosinase inhibitory activity

No antityrosinase activity was found at a tested concentration of 200 µg/mL for *A. bojeri, A. buchananii, C. edulis, C. galpinii, C. molle, C. velutina, C. burkeana, C. schinzii, F. burkei, H. procumbens, H. pallidum, H. hemerocallidea, I. crassipes, L. nepetifolia, O. sphaerocarpa, P. angolensis, S. transvaalensis, S. madagascariensis, S. cupulare* and *T. hypericoides*. Additionally, no previous reports on the antityrosinase activity has previously been reported for the plants investigated in this study, except for *A. nilotica* and *C. molle*. In the current study, *A. nilotica* showed an IC_{50} value of 12.97 ± 1.07 µg/mL, which was comparable to results obtained in a study by Muddathir et al. (2017), where *A. nilotica* showed an IC_{50} value of 8.61 µg/mL on the enzyme monophenolase tyrosinase (Muddathir et al., 2017). On the contrary, *C. molle*, with an IC_{50} value >200 µg/mL, differed significantly to the IC_{50} value of 126.2 ± 0.4 µg/mL reported previously (van Staden et al., 2017). There are several factors that may affect levels of biologically active compounds which affect quality and consistency of extracts. These include the age of plant, ecophysiological factors, geographical distribution, genetic variations, seasonal variation and post-harvest processing (drying, handling and storage) of material (Bopana and Saxena, 2007; Buwa and Van Staden, 2007; Street et al., 2008). The difference in the antityrosinase activity could potentially be a result of varying storage conditions of the plant material of the extract as well as the age of the plant extract. The long-term storage of extracts has been shown to decrease the phenolic content (Tao et al., 2014). Phenolic compounds have previously been reported for their antityrosinase activity that led to reduced melanin content (Ali

et al., 2012). Furthermore, these phenolic compounds have been identified in the extracts which exhibited antityrosinase activity in the current study (Abeer, 2011; Abubakar and Majinda, 2016; Adewusi et al., 2011; Marquardt et al., 2017; Singh and Arora, 2009). The phenolic compounds could therefore, provide a possible explanation for the antityrosinase activity of the extracts which showed high inhibitory activity, however, future research would include quantifying the phenolic compounds in the reported extracts to make an evident conclusion. The extracts of *I. oblongata* exhibited low antityrosinase activity with an IC_{50} value of 190 ± 1.20 µg/mL. The extracts which showed significant antityrosinase activity, with IC_{50} values substantially lower than the IC_{50} values obtained in the cytotoxicity assay, were *C. collinum*, *P. rotundifolius*, *S. brachypetala* and *V. infausta*, with IC_{50} values of 47.92 ± 1.13, 124.40 ± 2.03, 35.07 ± 0.71 and 52.81 ± 1.17 µg/ml, respectively. This indicated that these extracts showed tyrosinase inhibition at concentrations that would not exhibit toxic effects on the keratinocyte cells.

3.4 Antimicrobial susceptibility of Cutibacterium acnes to extracts

As described by Holetz et al. (2002) noteworthy antimicrobial activity was attributed to extracts with an MIC < 100 µg/mL, while extracts with an MIC between 100-500 µg/mL were considered as moderately active. The *H. procumbens* extract exhibited the highest antibacterial activity against *C. acnes* with an MIC of 31.25 µg/mL. However, based on the antiproliferative effect of the extract on keratinocyte cells, extensive use of this extract in a topical form should be exercised with caution. A study by Weckesser et al. (2007) reported the antibacterial activity of the aqueous extract of *H. procumbens* against several aerobic and anaerobic microorganisms associated with dermatological disorders. The MIC of the extract against three *Staphylococcus aureus* strains, including a methicillin-resistant strain, was 10 µg/mL. The extract also exhibited antibacterial activity against another microorganism associated with acne progression,

Staphylococcus epidermidis, with an MIC of 10 µg/mL. The *H. procumbens* extract showed lowered activity against another *C. acnes* strain (FR 024/12-10) with an MIC of 100 µg/mL. The results correlated well with the activity in the present study, and differences in activity could potentially be as a result of the FR 024/12-10 strain being a clinical isolate (Giannopoulos et al., 2015).

The extract of *S. brachypetala*, which has reported ethnobotanical use as a treatment for pimples, had an MIC of 125 µg/mL. The *in vitro* antibacterial activity against *C. acnes* therefore, validated the ethnobotanical use documented by Van Wyk and Gericke (2000). The organic (dichloromethane: methanol) and aqueous bark extracts of *S. brachypetala* were reported against *C. acnes* (ATCC 11827) with an MIC of 250 and 500 µg/mL, respectively (Nciki et al., 2016). The extracts of *C. collinum*, *O. sphaerocarpa* and *P. rotundifolius* showed similar activity with an MIC of 250 µg/mL, while *A. nilotica* inhibited bacterial growth at the highest concentration with an MIC of 500 µg/mL. The antibacterial activity of the aqueous and methanolic leaf extract of *C. collinum* was reported by Cock and Van Vuuren (2015) against the Gram-positive cocci, *S. aureus* and *S. epidermidis*. The methanolic extract showed better activity with an MIC of 330 and 317 µg/mL against *S. aureus* and *S. epidermidis*, respectively. However, it is important to note that the MIC of this extract surpassed the cytotoxic IC_{50} concentration and should be further investigated for safety if it is to be incorporated into a topical dosage form. A study by Sibandze et al. (2010) reported the antibacterial activity of *O. spaerocarpa* against *Escherichia coli* with an MIC of 1.20 mg/mL, while the combination of this species with *Syzygium cordatum* reduced the MIC to 0.33 mg/mL. The extract could therefore exhibit increased antibacterial activity when used in combination with other species, rather than a monotherapy. This is the first report for the activity of *P. rotundifolius* against the acne causing bacterium, *C. acnes*.

4. Conclusion

This study revealed the effective tyrosinase inhibitory potential of *A. nilotica, S. brachypetala* and *C. collinum*. Additionally, these extracts showed strong antioxidant activity against the DPPH free radical. Furthermore, these plant extracts showed moderate toxicity on the keratinocyte cells (IC_{50} >100). In a study by Kishore et al. (2018), the significant tyrosinase activity of a plant extracts was linked to the strong antioxidant activity as antioxidant compounds have the potential to inhibit molecules associated with melanogenesis. Furthermore, antioxidant compounds could potentially bind to the active copper site present within the tyrosinase enzyme, thereby inhibiting the enzyme (Briganti et al., 2003; Ebanks et al., 2009; Karg et al., 1993). Six extracts showed antibacterial activity against *C. acnes*, however only three of these extracts namely; *O. sphaerocarpa*, *P. rotundifolius* and *S. brachypetala* showed moderate to low antiproliferative activity against the keratinocyte cells. Furthermore, these extracts showed strong antioxidant activity adding to the anti-acne potential due to the role of reactive oxygen species in progressing acne by upregulating inflammation (Portugal et al. 2007). This study has identified several lead extracts with numerous potential applications in the cosmetic field, however, further investigation into possible mechanisms of action should be pursued.

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Conflict of interest

The authors have no conflict of interest to declare.

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